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Experimental evaluation of birds as disseminators of the cosmopolitan tick *Rhipicephalus sanguineus* (Acari: Ixodidae)

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HIGHLIGHTS

- ▶ Chicks are not suitable hosts for *Rhipicephalus sanguineus* ticks.
- ▶ Chicks would be unable to maintain *R. sanguineus* tick populations.
- ▶ *R. sanguineus* ticks may be delivered by chicks to new settings.
- ▶ Birds may fortuitously introduce *R. sanguineus* ticks into non-infested dog settings.

GRAPHICAL ABSTRACT

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ABSTRACT

Rhipicephalus sanguineus is believed to be the most widespread tick species of the world and its dissemination seems to rely on the diffusion of its main host, the dog. Empirical observations indicate that several bird species in urban areas regularly steal dog food. Such circumstances create a chance for *R. sanguineus* ticks to climb on birds and carry ticks to another site. In this work we evaluated experimentally the likelihood of birds (chicks) to either feed and/or carry *R. sanguineus* ticks from an infested site to another and to infest a host (rabbit) in the new location. Chicks were not suitable hosts for *R. sanguineus* ticks. Not a single adult tick engorged on chicks, yield as well as weight of engorged larvae and nymphs were very low and feeding period of these ticks was very long. However, a few larvae and, chiefly, nymphs were delivered to a new location either mechanically or after attachment and engorging total or partially on chicks. A few of these ticks fed successfully on rabbits. Further evidence on the capacity of birds to introduce *R. sanguineus* into non-infested dog settings should be provided by systematic examination of birds from urban areas, close to tick infested households.

1. Introduction

Among the approximately 896 known tick species (Guglielmone et al., 2010) *Rhipicephalus sanguineus* is believed to be the most widespread of the world (Pegram et al., 1987; Walker et al., 2000). The dissemination of this tick is attributed to its host preference, the dog, as well as capacity to maintain its whole cycle within human dwellings (Guglielmone et al., 2006). The taxonomic status of this species is not clear (Szabó et al., 2005) and possibly dog and human dwelling associated *Rhipicephalus* ticks are in fact a complex of closely related species.

Whatever the case, *R. sanguineus* ticks are important pests; dog infestations are many times unbearable and they also transmit major canine infectious agents such as *Ehrlichia canis* and *Babesia canis* (Walker et al., 2000). The proximity of dog increases the likelihood of human tick-biting and of the transmission of zoonosis as well. Thus severe zoonotic disease agents such as *Rickettsia conorii*

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(Parola et al., 2009) and *Rickettsia rickettsii* (Demma et al., 2005) may be transmitted by *R. sanguineus* to man.

It is generally believed that *R. sanguineus* tick dissemination occurs when infested dogs are taken or wander to new settings. If the new site proves to be adequate for the off host tick cycle and dogs regularly use it to rest, a new infestation site is established. Another possible way of dissemination is the one that occurs among houses with ticks climbing the walls and infesting neighboring dogs (Guglielmone et al., 2006).

The widespread dissemination of *R. sanguineus* makes us wonder whether this tick species might have other ways of spreading, such as, for example, birds carrying ticks from an infested site to another. Such form of dissemination is known to occur for a few tick species and tick-borne pathogens on migrating birds (Brinkerhoff et al., 2009; Jameson et al., 2012). Even though reported rarely, *R. sanguineus* ticks were already found on birds (Diogo et al., 2003; Szabó et al., 2008; Luz et al., 2012). In this regard an empirical but interesting observation was done by the authors; several bird species such as pigeons, thrushes, sparrows regularly steal dog food. Such circumstance creates a chance for *R. sanguineus* ticks to climb on birds and these hosts might either suffer parasitism or carry ticks to another site. Thus in this work we evaluated experimentally the likelihood of birds to either feed and/or carry ticks from an infested site to another and to infest a host in the new location.

2. Material and methods

2.1. Parasites

Two to four weeks old *R. sanguineus* ticks from a laboratory colony fed on tick-bite naïve rabbits and derived from engorged females picked from dogs of Uberlândia, Minas Gerais, Brazil were used.

2.2. Hosts

Seven day old chicks (*Gallus gallus*) represented birds and 6 month old rabbits (*Oryctolagus cuniculus*) represented dogs as hosts for *R. sanguineus*. All hosts were tick-bite naive at the beginning of experiments. For experiments hosts were maintained in cages either individually (rabbits) or in groups of six animals (chicks) in the animal facilities of the Ixodology Laboratory of the Federal University of Uberlândia. During this period host were fed with commercial ration and water "ad libitum" and kept at 22–26 °C.

2.3. Experimental design

Chick suitability as hosts for *R. sanguineus* ticks was evaluated in two experiments and this host's capacity to deliver ticks from an infested site to another was tested in additional two. In all experiments hosts were held in cages (chicks – 24,5 × 45 × 33 cm; rabbit – 46 × 46 × 46 cm) placed on white plastic trays (54 × 47 × 11 cm) that had borders surrounded by double-sided adhesive tape to allow recovery of engorged and unfed ticks. All experiments were evaluated and approved by the Animal Experimentation Ethics Committee of the Federal University of Uberlândia. Permits and Approvals (n^0 25/08) are on file in the office of M.P.J.S.

2.3.1. Experiment 1

Suitability of chicks as hosts for *R. sanguineus* tick larvae and nymphs was evaluated by releasing ticks inside feeding chambers glued to the hosts. Each chick, six per group, was infested with 40 larvae within each feeding chamber. Another group of six chicks was infested with 10 nymphs each animal. This experiment was executed four times. In the first two, plastic feeding chambers (~1 cm of diameter) were glued (Bracoplast®) to the top of the head of chicks whereas in third and fourth a small cotton beg attached with adhesive tape to the wings of birds was used to keep ticks. Every day feeding chambers were inspected and engorged ticks collected.

2.3.2. Experiment 2

Suitability of chicks as hosts were evaluated with infestations without feeding chambers. Ten couples of *R. sanguineus* adults as well as 40 nymphs and 100 larvae were released on the back of each of six chicks. Tray underneath the cage with six chicks was inspected daily and engorged ticks collected. This experiment was repeated two more times.

2.3.3. Experiment 3

Capacity of chicks to deliver *R. sanguineus* from an infested site to a host (rabbit) kept at another site was evaluated with chicks staying for short periods at each site. Briefly, a group of six chicks was placed for 10 min in a cage infested moments before with 3000 larvae, 500 nymphs and 50 adult couples and put in a plastic box (34 × 30 × 49 cm). Chicks were then placed in a second cage with one rabbit for 10 min. This procedure with the same animals was repeated two more times but without additional ticks. Rabbits then remained in the second cage whereas chicks were taken to a third cage and all hosts and environment (trays underneath cages) were observed for ticks for eight days. This experiment was repeated two more times, each time with a new parasites and hosts.

2.3.4. Experiment 4

Capacity of chicks to deliver *R. sanguineus* from an infested site to a host (rabbit) kept at another site was evaluated with chicks staying for one long period at each site. Briefly, chicks were placed in cage infested with 3000 larvae, 500 nymphs and 50 adult couples for 24 h only once. Chicks were then placed in a second cage for 24 h and were then taken to a third cage. A rabbit was placed in the second cage immediately after chicks were taken away and all cages and trays beneath them as well as the animals were inspected for ticks for eight days. This experiment was repeated two more times, each time with a new ticks and hosts.

2.4. Data analysis

Host suitability as well as tick transfer between cages were evaluated determining engorged tick recovery rate (tick yield) from each host (experiments with chambers) or from environment/trays and cages (experiment without chambers) and tick feeding and reproductive parameters as described elsewhere (Olegário et al., 2011). Data from repetitions of the same procedures were pooled and presented as means ± standard deviation. Highly unequal and many times too small size of samples precluded statistical analysis.

3. Results

3.1. Experiment 1

Yield and molting rate of larvae (Table 1) was very low for ticks fed on the head of chicks (0.62% and 25%, respectively) and higher on ticks from wings (8.12% and 75.5%, respectively). Under similar conditions, nymphs had a much better performance (yield and molting rate above 20% and 98%, respectively) and provided similar parameters whether attached to the head or wings (Table 2).

Table 1
Biological parameters of *Rhipicephalus sanguineus* tick larvae released on chicks either inside feeding chambers, on the head or wings, or without chambers. Results are presented as means ± standard deviation and range is shown between brackets.

Location of chamber	Yield (%)	Weight (mg)	Feeding (days)	Molt (days)	Molt (%)
Head (n = 12)[a]	0.62 ± 1.55 (0.00–5.00)	0.23 ± 0.02 (0.20–0.25)	6.0 ± 0.0 (6.0 – 6.0)	11.0 ± 0.0 (11.0–11.0)	25.0 ± 35.4 (0.0–50.0)
Wings (n = 12)	8.12 ± 8.60 (0.00–32.5)	0.18 ± 0.06 (0.10–0.26)	4.9 ± 0.5 (3.0–6.0)	10.5 ± 1.8 (8.0–14.0)	75.5 ± 32.5 (0.0–100)
Without (n = 3)	0.22 ± 0.39 (0.00–0.67)	0.12 ± 0 (0.12–0.12)	5.0 ± 0.0 (5.0–5.0)	12.0 ± 0.0 (12.0–12.0)	75.0 ± 0.0 (75.0–75.0)

[a] n refer to either chick numbers or to tray numbers when ticks were not restricted by feeding chambers.

Table 2
Biological parameters of *Rhipicephalus sanguineus* tick nymphs released on chicks either inside feeding chambers, on the head or wings, or without feeding chambers. Results are presented as means ± standard deviation and range shown between brackets.

Location of chamber	Yield (%)	Weight (mg)	Feeding (days)	Molt (days)	Molt (%)
Head (n = 12)[a]	20 ± 20.8 (0.00–60.00)	1.45 ± 0.06 (0.70–2.10)	5.9 ± 1.3 (4.0–9.0)	17.0 ± 1.6 (14.0–21.0)	100 ± 0.0 (100–100)
Wings (n = 12)	20.8 ± 21.5 (0.00–80.00).	1.38 ± 0.38 (0.88–2.20)	5.4 ± 0.8 (4.0–7.0)	16.3 ± 1.2 (15.0–19.0)	98.9 ± 4.0 (87.5–100)
Without (n = 3)	0.56 ± 0.24 (0.42–0.83)	1.57 ± 0.77 (0.70–2.40)	6.3 ± 1.5 (5.0–8.0)	13.3 ± 1.0b (12.0–14.0)	100 ± 0.0 (100–100)

[a] n refer to either chick numbers or to tray numbers when ticks were not restricted by feeding chambers.

3.2. Experiment 2

Very few larvae and nymphs engorged on chicks (yield of 0.22% and 0.56%, respectively) if not protected by feeding chambers (Tables 1 and 2) and not a single adult tick from the 180 couples released on 18 chicks engorged. In fact, hosts were seen to ingest many ticks from their own body and also from the mates. Furthermore, many unfed ticks (1 adult, 8 nymphs and 19 larvae) were found trapped in the double sided adhesive tape of trays.

3.3. Experiments 3 and 4

Not a single adult female *R. sanguineus* and only a small proportion of larvae and nymphs engorged on rabbits after delivery by chicks from an infested site (Table 3). Tick delivery after repeated short permanence periods (3 × 10 min) of chicks in an infested site yielded only nine engorged larvae (0.09%) on rabbits whereas a single longer permanence of chicks at the infested site (24 h) yielded 18 engorged larvae (0.20%) on rabbits. In similar conditions nymph yield on rabbits was of 33 engorged nymphs (2.20%) after the short permanence periods of chicks and of 8 engorged nymphs (0.53%) after the longer permanence of chicks at the infested site.

Following tick delivery to rabbits, chicks were taken to a third tray and a small proportion of larvae (n = 4/0.03%) and nymphs (n = 3/0.2%) engorged on these chicks but only on hosts that stayed for short periods (3 × 10 min) at the tick source site (Table 4).

Several ticks, mostly unfed, were trapped by double sided adhesive tapes of trays in these experiments. In experiment 3, 45 unfed ticks (4 adults, 8 nymphs and 33 larvae), were found trapped at the rabbit trays. In the same experiment 44 unfed ticks (3 adults, 10 nymphs and 31 larvae) were trapped on trays where chicks remained for eight days. In experiment 4, 2 unfed (1 adult and 1 nymph) and five engorged larvae (all damaged by manipulation) were found trapped on rabbit trays and 1 unfed tick (nymph) was trapped on trays where chicks remained for eight days.

4. Discussion

Our experiments showed that chicks are not suitable hosts for *R. sanguineus* ticks. Not a single adult tick engorged on chicks, yield of larvae and nymphs was very low, feeding period was long and weight of engorged ticks low if compared to data from previous reports from tick feeding on guinea pigs (Bechara et al., 1995) or rabbits (Szabó et al., 2005). At the same time, the higher yield of larvae from chambers on wings in relation to head of chicks indicates that feeding site on host may interfere with tick development, an issue to be further investigated. It was also noted that tick-predation of chicks is very efficient and tick yield was lower when such host behavior was not prevented by feeding chambers. Concomitantly, ticks were not much attracted by chicks and many wandered away and were trapped by the adhesive tape. Thus it can be supposed that chicks would be unable to maintain *R. sanguineus* tick populations on their own.

Regarding the capacity of chicks to carry viable ticks from an infested site, it was observed that very few ticks fed on hosts (rabbits) at the delivery site. In fact, not a single adult engorged on rabbits whereas only a few immatures engorged on rabbits after either short or long permanence intervals of the transporter host at the infested site. Lack of adult ticks in the process might be attributed to their larger size which turned them into easy prey for chicks. Furthermore, movement of adult ticks was probably more sensed by chicks and elicited self-cleaning behavior more readily. At the same time, yield of nymphs was repeatedly higher over larvae in the various experiments and thus proved to be the

Table 3
Biological parameters of *Rhipicephalus sanguineus* tick larvae and nymphs engorged on rabbits and delivered by chicks infested in an environment after three exposures of 10 min or one of 24 h. Results are presented as means ± standard deviation and range shown between brackets.

Exposure periods	Yield (%)	Weight (mg)	Feeding (days)	Molt (days)	Molt (%)
Larvae					
3 × 10 min (n = 3)[a]	0.09 ± 0.10 (0.00–0.20)	0.18 ± 0.04 (0.10–0.20)	3.9 ± 0.6 (3–5)	11 ± 0 (11–11)	75.0 ± 35.4 (50.0–100)
24 h (n = 3)	0.20 ± 0.20 (0.00–0.40)	0.26 ± 0.06 (0.18–0.30)	3.3 ± 1.8 (2–6)	11.5 ± 0.9 (11–13)	91.7 ± 11.8 (83.3–100)
Nymphs					
3 × 10 min (n = 3)	2.20 ± 2.80 (0.40–5.40)	2.80 ± 0.4 (1.60–3.40)	4.8 ± 0.8 (3–6.)	14.5 ± 0.8 (13–16)	91.7 ± 14.4 (75.0–100)
24 h (n = 3)	0.53 ± 0.31 (0.20–0.80)	2.66 ± 0.34 (2.00–3.00)	4.0 ± 1.9 (2–6)	14.8 ± 0.7 (13–15)	100 ± 0 (100–100)

[a] n refer to tray numbers.

Table 4
Biological parameters of *Rhipicephalus sanguineus* tick larvae and nymphs engorged on chicks on third tray after being used to deliver ticks to rabbits from an infested site after three exposures of 10 min or one of 24 h. Results are presented as means ± standard deviation and range shown between brackets.

Exposure periods	Yield (%)	Weight (mg)	Feeding (days)	Molt (days)	Molt (%)
Larvae					
3 × 10 min (n = 3)[a]	0.03 ± 0.06 (0–0.10)	0.15 ± 0.03 (0.10–0.16)	4.5 ± 1.0 (4–6)	11.3 ± 0.5 (11–12)	100 ± 0.0 (100–100)
24 h (n = 3)	0.00	–	–	–	–
Nymphs					
3 × 10 min (n = 3)	0.20 ± 0.35 (0–0.60)	2.67 ± 0.12 (2.60–2.80)	6.3 ± 0.6 (6–7)	14.3 ± 0.6 (14–15)	100 ± 0.0 (100–100)
24 h (n = 3)	0.00	–	–	–	–

[a] n refer to tray numbers.

most suitable stage for both feeding on chicks as well as delivery to rabbits.

A few larvae and nymphs engorged on rabbits as fast as in two days in the fourth experiment (24 h of chicks at the infested site). This feeding period is very short for *R. sanguineus* (Szabó et al., 2005) and probably some of these ticks fed initially on chicks, detached partially engorged and finished engorgement on rabbits. Moreover compared to data from chick infestations without chambers, engorged larvae and nymphs were heavier after feeding on rabbits. These observations suggest *R. sanguineus* preference for rabbits as hosts over chicks.

Taken together observations above indicate that chicks are not easily infested even at environment with high tick density. However, those few ticks that climb chicks, if not swallowed by host, may be delivered to a new setting either mechanically or after attachment and feeding total or partially on chicks. In this sense unsuitability, albeit decreases infestation of hosts at the source site, enhances delivery at a new site by facilitating tick detachment, particularly if a better host is provided.

Although this tick species prevalence as well as dog infestation intensity varies over the world, it was estimated that at a given time point around 37% of dogs from Uberlândia, Minas Gerais, Brazil, harbor *R. sanguineus* ticks from which 3% are highly infested (Szabó et al., 2010). This means that at least 910 from the estimated 82,000 population of dogs from this municipality are associated with high levels of environmental tick infestation. Hence, in a natural scenario, some basic requirements for bird delivery of ticks are established in Brazil. It is a tropical and sub-tropical country with weather permissive for *R. sanguineus* infestation all over the year, there are several *R. sanguineus* spots in urban areas with high infestation levels and urban avifauna is rich (Franchin and Marçal Júnior, 2004). Thus regular daily visit of birds to even a few of this settings provide conditions for *R. sanguineus* and bird contact. Similar conditions are likely to occur in other tropical countries or summer time in temperate countries.

Curiously fulfillment of some of these requirements has recently been observed by Luz et al. (2012) in a rural/natural area interface. During a survey of bird tick infestations at the Ecological Station Pirapitinga-ESEC from Minas Gerais State, Brazil, these authors collected seven *R. sanguineus* nymphs (that molted to adults) from seven (13.5%) out of 52 individuals of single passerine bird species (*Gnorimopsar chopi*). Furthermore the authors observed in a rural area close to bird capture site, a group of 15 *G. chopi* feeding on the ground next to dogs. The authors considered this last location as the probable site for *R. sanguineus* infestation of birds.

Still, a few additional variables should be addressed to evaluate bird delivery of *R. sanguineus* ticks. The major one is the differing suitability that might be found among diverse bird species to *R. sanguineus* ticks, especially of those synanthropic. Secondly the regularity of individuals of these bird species to visit dog settings as well as their behavior at such location should be known in further details. Frequency of bird visits to several or at least two dog settings in a row to permit tick the delivery should also be evaluated. Last but not the least behavior of ticks to attach to and detach from various bird species is also an important issue to address in this context.

Finally it must be stressed that our experiments should be viewed with some caution; chicks might not have been an adequate representative for bird species and the scenario with chicks, rabbits and ticks may have been too unusual. Moreover considering data from this work, it is clear that should birds disseminate *R. sanguineus*, it is rather a rare than a massive and/or constant event.

Further evidence of this issue could be given by systematic examination of birds from urban areas, close to tick infested households. Given the cosmopolitan distribution and relevance of *R. sanguineus* as infectious disease vector, such research is worthwhile.

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